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Some Problems of Tissue Heterotransplantation.

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The problem of organ and tissue transplantation is acquiring greater and greater actuality at the present time. The improvement of technical equipment, the refinement of the operational technique, the development of anesthesiology and the broad use of antibiotics, are all contributing to an enlargement of the possibilities of plastic surgery.

There is no doubt that the best plastic material is the autograft. Autoplasty, however, does not completely fill the requirements of restorative surgery. Also, it is often impossible to use a fresh homograft. It is not always possible to take the necessary tissue from a living person without damage to the well being, and, moreover, a fresh homograft adapts more poorly and disengages more rapidly.

The development of modern surgery requires large supplies of various plastic materials having prolonged storage periods without loss of their biological function.

Homoplasty owes its successes to the works of V. N. Shamov (1929), who first proved the possibility of transfusing corpse blood taken from unexpectedly deceased individuals. These works also opened wide perspectives for the transplantation of solid tissues taken from a corpse. This, alone, created a real possibility to secure large reserves of homografts.

In 1947, the Leningrad Institute of Blood Transfusion was the first to organize a center for the preservation and transplantation of tissues. Later, they also began to open "tissue preservation banks" abroad. The acquisition of homograft reserves, however, has still not solved the problem of homotransplants. Homografts transplanted from one person to another do not adapt because of the existence of tissue incompatibility.

The causes of death of the transplants continue to interest scientists of many specialties (biologists, surgeons, embryologists, biochemists, physiologists et al) and still are not precisely defined. The majority of investigators believes that the homo-heterograft dies as a result of a deep difference

in the biological properties of the tissues and fluids of the donor and the host, and the development of specific antibodies against the transplant in the host. In other words, the fate of the transplant depends on the immunological reaction.

Complicated biological processes develop in both the host organism and in the transplant in any free transplantation of organs and tissues. In the transplant, there occur unavoidable degenerative changes that are related to the disruption of innervation and blood supply. These changes are then replaced by the regenerative processes in autoplasty, and by a displacement of the transplant by the host's tissues in homoplasty and heteroplasty. The reactive changes in the surrounding tissues contribute to the adaptation of the transplant. In homo- and heterotransplantations, the reactions of the tissues and of the entire organism consist of a generation of antibodies in response to the tissular antigens of the transplant, which leads, in the final analysis, to the disengagement or resorption of the latter.

Inasmuch as the fate of the transplants depends on immune reactions, the question of tissue compatibility has an immunological basis and represents a general biological problem.

According to G. V. Golovin (1959), the interrelation of the host's immune system with the transplanted graft is as follows: "The host organism responds to the introduction of the extraneous tissue (antigen) with an expressed allergic reaction and a production of antibodies, which concentrate in the blood stream. During the interaction of the antigens with the antibodies, there are histaminelike substances released which, upon the restoration of vascularization, exert their destructive effect on the transplant and, by this, prevent its adaptation."

There is no doubt that in time it will be possible to overcome the biological incompatibility of the tissues and elucidate the conditions necessary for the successful adaptation of homo-and heterotransplants. This is shown by experiments on immunological rapprochement and on acquisition of host tolerance to the donor's tissues (M. Gashek, A. Lengerova et al), and also by the successful adaptation of tissues in monozygotic twins.

Since, at the present time, we are still unsuccessful in achieving true adaptation of homografts, it is necessary that we channel all of our efforts toward slowing the disengagement of the transplant.

Stemming from these considerations, the scientific development of tissue transplantation has been conducted mainly in two directions:

1. An effect on the host organism for the purpose of depressing the reaction to the antigen.

2. An effect on the transplant for the purpose of depressing its antigenic properties.

There is a great number of works related to weakening the immunological reactivity by the effect of X-rays on the host organism (Dempster et al, 1950; K. M. Akylbekov, 1954; A. N. Mazaev and P. M. Chepov, 1953; Hardin and Werder, 1955), by medication sleep (M. I. Yefimov, 1952; Sh. V. Musina, 1952, 1953, et al), by repeated homoplastic transplantations over set periods of time (Werder and Hardin, 1954), by massive total-replacement transfusion of blood to donors and hosts (P. I. Kosyakov and P. M. Chepov, 1955, et al), by the use of acute total plasmophoresis on the host (P. M. Chepov, 1953, et al), by blockades of the reticuloendothelial system with injection of the body (Morpurgo and Milone), by starvation and emaciation of the patient (Ye. R. Kolpakova), by sympathectomy (Uffreduzzi, Placintanu, 1924; Sazon-Yaroshevich, 1932-1937) and by many other measures.

Regardless of the success of some of these methods in prolonging the survival of homotransplants, they still have not found acceptance in clinical practice and are still in the category of experimental investigations.

The most perspective at the present time is the investigation of methods for the preservation of tissues, which would permit a decrease, or elimination of the transplant's immunobiological activity.

It has been proved by experimental investigations and clinical observations that the best method is preservation by freezing at a certain regime, and also by lyophilization.

Prolonged preservation of the transplants improves the results of homotransplantations. The transplant adapts with the mildest tissular reaction on the part of the organism.

According to many authors (A. A. Vishnevskiy, 1959; Vamio; Salonen, 1957; Kubanyi, 1958), the cold and dehydration, while depressing the antigenic properties of the transplant, kill the tissue at the same time. A dead graft that is transplanted is similar to a prosthesis.

Other authors (M. V. Bilenko, M. M. Kapichnikov, 1957) believe that the freezing and dehydration, in themselves, will not cause weakening of the antigenic properties of the tissues.

Dehydrated transplants can be stored for a long time and subjected to the effect of high temperature with the retention through this of desirable properties and the elimination of some of the harmful.

One of us (M. A. Lushchitskiy), during 1955, conducted an analysis of the changes in the antigenic properties of human skin, preserved in the cold at a temperature of +4° to+6°C, with a simultaneous histological investigation of it. It was established, by use of the specific iso-agglutinin absorption reaction, that the antigenic properties of the skin, beginning from the 15th-20th day of preservation, are lowered, but do not disappear completely over a one-month period. Skin that has been preserved for a one-month period at a temperature of +4° to +6°C, retains its structure and properties, and can be successfully used for transplantations.

The lack of a simple and practicable apparatus for freezing and dehydration, the complexity of these processes, the difficulty of prolonged storage, transportation and establishment of large supplies of homografts, all impede, to a certain degree the wide use of homografts.

Heterogenic tissues have recently begun to be used for plastic surgery (peritoneum, skin, bone, cartilage, vessels, etc).

Heteroplasty has been known from olden times, although the first unsuccessful attempts at clinical use (Ollier, 1860; Barth, 1895; N. I. Bashkirtsev, 1911; N. N. Petrov, 1912; Lexer, 1929; et al) long retarded the development of this form of plastic surgery.

The use of fresh heterotransplants is doomed to failure because of their expressed antigenicity and greater protein incompatibility. Heterografts that are preserved by freezing and also lyophilized, just as the homografts, cannot find wide acceptance because of the same reasons. Therefore, investigations should be aimed either toward a rapprochement with the host's tissular qualities, which at present is still difficult, or toward a search for tissues that have no expressed antigenic properties.

Influenced by the works of O. M. Zemtsova and A. A. Terekhova (1928), in which was demonstrated the lack of group antigenic properties in fetuses up to the age of six months, and by the communications of many authors (A. N. Okulova, 1951; G. I. Gintsburg, 1951; G. V. Lopashev and L. M. Dykman, 1953; et al) about the more expressed stimulant properties and plasticity of fetal tissues, the coworkers of the chair, upon the proposal of Prof. A. A. Bocharov, have been investigating (since 1954) the possibility of the practical use of heterotransplants from calf fetuses (vessels, bone, skin). In 1951, Rogers used the skin of a calf fetus as a temporary biological bandage and in this noted that resorption of the transplant occurred after 16 days. The transplant contributed to the development of granulation and epithelization. In 1957, Rogers, Converse and Silvetti communicated about a heterotransplantation of calf-embryo skin to humans and indicated an absence of noticeable sensitization in the hosts.

Each method can find wide acceptance only when it is simple and generally practicable.

Inasmuch as antigenic properties are not expressed in fetal tissues, we adopted the simplest and most generally practicable methods of preserving the transplants.

K. M. Lisitsyn, using A. P. Nadein's idea, developed a method for the preservation of bone transplants in paraffin at room temperature. The establishment of large reserves of heterogenic bone transplants from fetuses, with a storage period of over 12 months, was proved possible on the basis of bacteriological and histological investigations of the transplants and by experimental transplantation of them on dogs and in the clinic.

The bone transplants that are preserved up to four months in the paraffin possess the greatest biological activity. It was established that for the best

results of heteroplasty, it is necessary to use perforated transplants and to strive for their intimate contact with the bone and soft tissues of the host over a large surface. Heterografts that are transplanted into a bone defect are subjected to change, resorbed and are replaced by newly formed tissue.

In our experiments, we succeeded in observing, in ocular demonstration, the process of slow resorption of heterotransplants from fetuses and the replacement of them by the newly formed tissue of the host. In these, we used the micro- and macrofluorescent method (M. A. Lushchitskiy, K.M. Lisitsyn, A. K. Revskoy). An absence of early absorption of the heterotransplants in repeated transplantations proves that the tissues from fetuses possess no expressed antigenicity. In addition to our study of the efficacy of replacing defects of tubular bones in experiment, we are also investigating the possibilities of replacing bone cavities and defects of the cranium with bones from fetuses (K. M. Lisitsyn).

The simplicity of preparation, preservation, storage and transportation, creates many advantages over the earlier methods.

After the experimental investigations, we used the bone heterotransplants from calf fetuses in clinical practice with satisfactory short-range results. The long-range results are as yet unknown.

The numerous experiments that have been conducted on the replacement of blood-vessel defects with a portion of artery from a mature animal have been unsuccessful in a large percentage of cases as compared with other types of vascular plastic surgery (Hoepfner, Carrel, A. I. Morozova, V. R. Braytsev, Oudot et al). The majority of the authors believe, however, that it is necessary to continue the search for heterovessels that will insure better results. Only a wide use of animal vessels can make possible the stockpiling of large supplies of vascular transplants that are free of sclerotic changes.

Our experiments on dogs (A. K. Revskoy) showed it possible to transplant vessels from 4-6 month calf fetuses, both fresh and also those preserved in N. G. Belen'ko's serum, in aminopeptide and in A. D. Belyakov's fluid.

A study of the histological preparations allows us to come to the conclusion that the vessels from the fetuses are replaced gradually by the tissues of the hosts, but this process occurs slowly. If the vascular heterograft is a bloodvessel wall with poorly differentiated layers, then by the sixth month of transplantation there is an evolution of all of the wall's layers to correspond to the layers of the host's vessel.

We were unable to detect any difference in the results of using fresh and preserved vascular transplants.

In clinical practice, we made repeated transplantation of cutaneous heterotransplants from 6 month old calf fetuses to two patients with ulcers of the shins, which were stubbornly resistant to healing. For a protracted time, these patients had been unsuccessfully treated by various methods and with repeated autotransplantations of skin. The heterotransplants from the fetuses were also resorbed and disengaged within 7-12 days after transplantation,

but meanwhile they contributed to a revival of granulation and to a resorption of the scars around the ulcers. A subsequent autoplasty of skin was successful. A durable complete adaptation of the flap occurred in both cases. There was no sensitization of the organism observed.

A check for changes in the immunological properties of the host's blood serum (in an experiment on rabbits and dogs), by the hemagglutination reaction, showed that heterotransplants of vessels, bone and skin of the fetuses (fresh and preserved) do not sensitize the organism of the host and cause no expressed immunological changes that can be detected by the iso-hemagglutination reaction. This causes us to believe that the tissues of embryos, as a result of slight differentiation, possess insufficient antigenic properties to produce the formation of antibodies in the organism of the host. At the same time, our experiments give basis to assume that the iso-hemagglutination reaction is not strictly specific for the detection of tissular (transplant) antibodies. Therefore, it cannot be used for the investigation of immunological shifts in the organism during heterotransplantation of grafts from fetuses.

The encouraging results make it possible to speak out concerning the advantage of a wider use of calf-fetus grafts in clinical practice and a broader scientific treatment of the heterotransplantation problem.

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